N. C.—4

FUSARIUM-BLIGHT, OR WILT DISEASE, OF THE SOYBEAN

BY

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Reprinted from JOURNAL OF AGRICULTURAL RESEARCH
Vol. VIII, No. 11 : : : : Washington, D. C., March 12, 1917

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1917

FUSARIUM-BLIGHT, OR WILT DISEASE, OF THE SOYBEAN[1]

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INTRODUCTION

During the summer of 1915 a package of diseased plants of the soybean (*Soja max* (L.) Piper (17);[3] syn. *Glycine soja, Soja hispida*, etc.)[4] was received from a correspondent at Red Springs, N. C. A large number of plants in the field from which these specimens were taken had become chlorotic, or were dead. The plants received were still green and in good condition for examination. The evidence obtained from a preliminary inspection indicated that the diseased condition was due to the presence of a fungus belonging to the genus Fusarium. Furthermore, nearly all of the isolations from this material gave pure cultures of a species of Fusarium.

The studies herein reported were therefore undertaken (1) to determine the parasitism of this species of Fusarium on soybean, (2) to establish its relationship to the Fusaria of the section Elegans in so far as a comparison of the cultural characteristics permit, and (3) by means of cross inoculations and field studies to determine the relationship of this disease of soybeans to the wilt disease of cowpeas (*Vigna sinensis* Hassk.) caused by *Fusarium tracheiphilum* Smith.

ECONOMIC IMPORTANCE OF THE SOYBEAN

The soybean is a native of tropical Africa, Asia, and Australia (23, p. 360–361; 17, p. 76) and was introduced into Europe by Kämpfer about 1690 (18, p. 9). At the present time it is the most important legume grown in Japan, China, and Manchuria. Its culture in England was begun in 1790. The plant was introduced into the United States from Japan in 1860. Since that time its cultivation as a soil-improving and a forage crop has been confined for the most part to the Southern States. North Carolina is probably foremost among these States in the production of soybeans. The yield in 1909 was 13,313 bushels,[5] and in 1915 was estimated[6] as approximately

[1] Published with the permission of the Director of the North Carolina Agricultural Experiment Station.

[2] The writer wishes to express his deep appreciation to Prof. H. R. Fulton, under whose direction the study was begun, and to Dr. F. A. Wolf, for his helpful suggestions and criticisms during the major part of the investigations and for aid in the preparation of the manuscript.

[3] Reference is made by number to "Literature cited," p. 438–439.

[4] For a complete synonymy, see Piper (17).

[5] U. S. Bur. Census, 13th Census, 1910, 1913. Statistics for North Carolina, p. 632.

[6] Estimate furnished by the North Carolina Experiment Station.

Journal of Agricultural Research,
Washington, D. C.
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Vol. VIII, No. 11
Mar. 12, 1917
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1,000,000 bushels. Within the last two or three years this crop has become increasingly important because of the variety of products manufactured from the oil and meal.[1] During 1915, $9,000,000 worth of oil alone was imported. Local cottonseed-oil mill owners have been induced, however, partially by the efforts of the North Carolina Experiment Station, to crush soybeans during their otherwise idle season. The few mills in the State which have done this have found a ready market for the oil and meal.

OTHER SOYBEAN DISEASES

Soybeans are very generally observed to be quite free from disease, and no very seriously destructive parasites of this host appear to have been reported in the literature at hand. Of those reported, a detailed study has not been made, except in the case of *Bacillus lathyri* Manns and Taubenhaus (13, 14). The accounts of the others consist of brief fragmentary mycological notes and mention of their place of collection or of their appearance. Since any of them may appear on plants affected with blight or wilt, it is deemed advisable to call attention to the published accounts of these diseases and the appropriate bibliography.

Septoria sojina v. Thümen (on living or declining leaves) (24).
Phyllosticta sojaecola Massalongo (15, p. 688).
Aecidium glycines P. Henn. (6, p. 52).
Uromyces sojae (P. Henn.) Sydow (22, p. 429).
Bacillus sp. (on leaves)—Heald (9, 10), Smith (21), and Clinton (4).
Bacillus lathyri Manns and Taubenhaus (on leaves and pods) (13, 14), and Manns.
Heterodera radiciola—Scofield (19, p. 9), Gilbert (8, p. 9), Bessey and Byars (2, p. 8). (These authors merely mention the soybean as a host for this parasite.)
Chlorosis and crinkling (cause?) (Description of the disease in the field)—Clinton (5).
Septoria glycines T. Hemmi (comparison with *S. sojina* above) (11).

It is not believed that the presence of any of these organisms would lead to confusion in the diagnosis of blight caused by the species of Fusarium under consideration.

HISTORY AND OCCURRENCE OF THE DISEASE

No published report of a disease of soybeans caused by any species of Fusarium and one account only of attempts to produce a disease of this host with the cowpea wilt organism have been brought to the writer's attention. Orton (16, p. 16–19) conducted these tests at Edisto Island, S. C., in 1900, and at Monetta, S. C., in 1901. Several varieties of cowpeas and soybeans were planted on soil badly infected with the

[1] The following is a list of the most important products obtained from soybeans or in which soybeans enter: Soybean milk, meal or flour, soups, pork and beans, meat substitutes, fertilizer and cattle feed from the meal, and dynamite and high explosives, soaps, linoleum, rubber substitutes, margarine, Japanese sauce, paints, varnishes, toilet powder, waterproof cloth, salad oil, lubricants, and lard substitutes from the oil.

cowpea-wilt organism and with a nematode (*Heterodera radicicola*). Concerning the work at Monetta, S. C., he says (p. 18):

> Eight varieties [soybeans] were tried on ten plats. All proved to be immune to the wilt disease, but none of them was adapted to the local conditions. The growth was very small, the plants averaging from 8 to 14 inches high, though most of the varieties bore a good crop of seed for such small plants. All suffered from much drought in midsummer and all were badly injured by the root nematode. On examination of the roots a moderate number of bacterial tubercles were found. * * * They [soybeans] were at a considerable disadvantage in this test on account of the late date of planting and the ensuing dry weather.

The varieties tested were Tokio, Buckshot, Yosho, Ito San, Manhattan, Guelph, and Amherst. Orton reported that at Edisto Island the soybean made a heavy growth, 3 or 4 feet high and was free from the wilt disease. It may be said that a very considerable proportion of the several varieties of cowpeas grown in adjacent plots succumbed to wilt. The results of these tests accord with the observations of others who have had opportunity to observe these crops when they were grown on soil known to be infected with cowpea wilt.

A limited number of careful observations have therefore been made during 1915 and 1916 to determine whether the wilts of these two hosts are coextensive in range and thus to furnish evidence of the identity of the two. Two 5-acre fields on widely separated parts of the North Carolina Experiment Station farm, in which cowpeas and soybeans were grown in alternate rows, showed a very considerable proportion of the former host affected, whereas the latter remained entirely free from disease. In other localities of the State, soybeans growing on soil infected with the cowpea-wilt organism have remained disease-free.

Observations differing from these were made in the case of soil brought from another part of the Station farm. When this soil was used to grow soybeans in pots out of doors, it was found to be infected with soybean-blight, as shown by the development of the disease in 33 of the 80 pots. Wilt, both of cowpeas and soybeans, was present on the farm of the correspondent previously referred to, at Red Springs, N. C. Many of the soybean plants in this field were killed and many only stunted, so that a decrease in yield of 60 per cent during the past season is probably a correct approximation of his loss. Blight or wilt of soybeans has also been found to occur at Exum and Belhaven, N. C., and was the cause of considerable loss in both locations.

Since cowpea-wilt has been found in many localities throughout the Piedmont and the Coastal Plain sections of North Carolina, it is entirely probable, if we judge from the results to be presented subsequently, that the soybean-blight may appear more or less generally wherever the soil is infected with *Fusarium tracheiphilum*. Records received from the Office of Plant Disease Survey show that, up to the close of 1915, *F. trachei-*

philum has been reported as being productive of losses to cowpeas ranging from 2 to 100 per cent in Indiana, Missouri, Mississippi, Louisiana, Texas, Oklahoma, Georgia, Florida, North Carolina, South Carolina, and Virginia.

APPEARANCE OF THE BLIGHT IN THE FIELD

In 1916, soybeans were planted during the last two weeks in May. This is somewhat later than usual, being due to the late season and a period of drought. When the plants were 4 weeks old, they had attained a height of 2 to 3 dm. and were apparently still free from disease. The disease was first observed on July 25, when the affected plants were about 8 weeks old. Symptoms of the trouble could probably have been found a week or two earlier. Affected plants, all of the same age but varying in height from 2 dm. to 1 meter, were observed on the 25th. The fungus is believed to have stunted these small plants. In no case has the disease been observed on seedlings.

The contrast in appearance of five healthy and five diseased plants is shown in Plate 95, *D*, *E*. The same type of clay soil was used in both pots, and the plants in each were grown out of doors under the same conditions. The plants shown in figure *E* were naturally infected from the soil. A considerable number of the leaves have fallen from the diseased plants, a portion of the petioles persist, the plants are dwarfed, and there is no evidence of wilting in any part of the plants. The foliage which persists on these plants is yellow as contrasted with the normal leaf green of healthy plants.

The occasional absence of a definite wilting of the leaves has been noted in other wilt diseases. Orton (16, p. 10), in speaking of the cowpea disease caused by *F. tracheiphilum*, says:

> The term "wilt" is somewhat misleading, as the leaves usually drop off before there is any conspicuous wilting. The name was applied because of its relationship to the wilt of cotton and watermelons, where this symptom is very prominent, and it seemed desirable to retain it for the cowpea disease.

In the case of the soybean disease, wilting is a less prominent symptom than in cowpeas, and is very seldom present at any stage of the disease. The plants, as a rule, drop all of their leaves and die without any evidence of wilting. Wilting has been observed in a very few instances in the field in the case of young plants. The woody nature of the stem and petioles probably accounts for the general absence of wilting in them, and the presence of well-developed mechanical tissues in the leaflets may account for their failure to manifest wilt. The possibility exists, also, that the physiological interaction of parasite and host differs from that exhibited by wilted cotton and watermelons infected with *Fusarium* spp.

Instead of applying the name "wilt," therefore, to the soybean trouble, it is perhaps desirable to call it "blight or wilt," the former

word describing the most prominent symptom on the foliage and the latter retaining the idea of its relationship to other wilt diseases produced by species of Fusarium.

Soybean blight or wilt may make its appearance on individual plants, but does not cause the death of all the plants within definite areas, as in the case of cotton wilt.

Although no definite effort has been made to determine the method of entrance of the organism into the host, it is thought that it enters through the smaller roots in practically the same manner as that described for other diseases of this character. Many of the fibrous roots are destroyed, and new roots are formed of insufficient number, however, to maintain the life of the plant.

Perhaps the most prominent symptom is a browning or blackening of the interior of the stems and roots. As soon as the leaflets begin to drop, this discoloration is evident when the root and stem are split longitudinally. This character is shown in Plate 95, figures *B* and *C*, showing healthy and diseased stems, respectively. As the disease progresses, the discoloration extends upward in the stem for one-half or more of its length. The tracheal tubes of affected stems when cut obliquely show as brown spots. The relative amount of discoloration in general and the depth of color in affected xylem portions is less in soybeans than in cowpeas.

A large number of stained free-hand sections were made of stems at all stages of development of the disease. In the early stage, only the xylem tubes nearest the pith were found to contain the fungus filaments. The pith had disappeared in both normal and diseased plants of moderate size. Later, other of these tubes throughout the xylem area are penetrated and become filled to a large extent with a network of fungus filaments. In still more advanced stages, all of the xylem elements (fig. 1, *A–G*) were found to contain the fungus; and, in addition, the cortical parenchyma was invaded.

The surface of stems of plants in advanced stages of the disease generally have salmon-colored spore masses, sporodochia, thickly and irregularly distributed over them.[1] This character is shown by the roughened appearance of the stem in Plate 95, *A*. The spore masses are composed of macroconidia of the fungus and are frequently found to occur on plants whose upper leaves are still healthy in appearance. Sometimes they are formed only in more advanced stages of the disease.

MANNER OF INFECTION AND SPREAD OF THE DISEASE

As stated above, the fungus may enter the plant through the small roots. In addition to the spread of the organism through the soil, spores are so abundantly produced that drainage water, implements,

[1] Sporodochia on stems of cowpeas are reported by Orton (16, p. 9) to appear after the death of the plants.

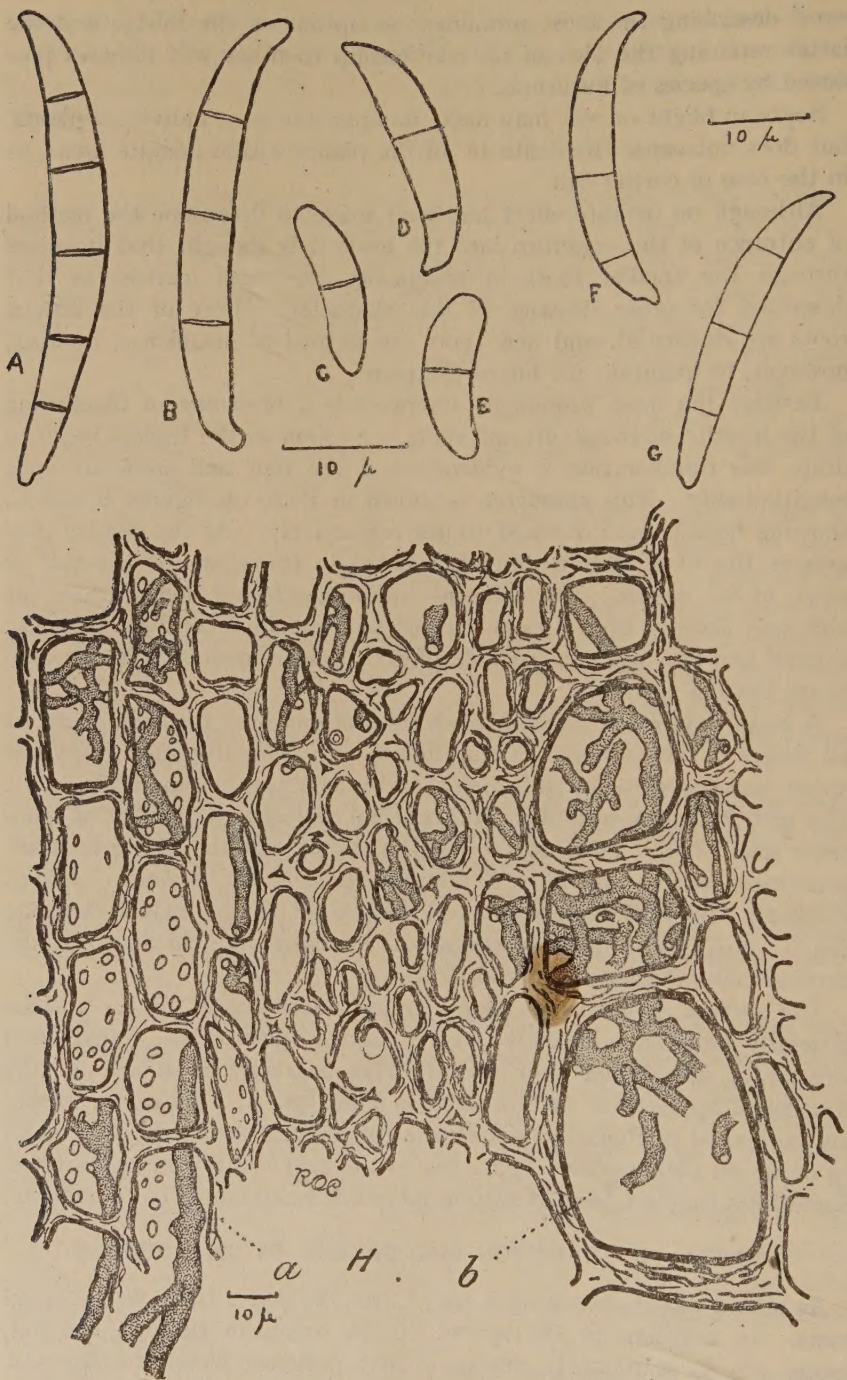

FIG. 1.—*A–G*, Types of macroconidia of the species of Fusarium on soybean. *H*, Cross section of the xylem portion of a diseased soybean stem, showing the invasion of the medullary rays (*a*) and the xylem vessels (*b*) by mycelia of the species of Fusarium on soybean.

and animals are also probably concerned in its spread. In all cases observed except one, nematodes have been present and probably facilitated the entrance of the fungus. The nematodes were found in infected sandy soil, but not in infected clay soil.

COMPARISON OF THE SOYBEAN SPECIES OF FUSARIUM WITH OTHER WILT-PRODUCING SPECIES OF THE GENUS

SOURCE OF CULTURES AND METHODS OF ISOLATION

Isolations were made from the interior of stems of freshly wilted soybean and cowpea plants. The stems were first thoroughly washed in water and allowed to remain wrapped in cotton moistened with 0.1 per cent solution of mercuric chlorid for 15 minutes. They were then split open so that the diseased interior was exposed. Fragments of diseased tissue were removed with a sterile scalpel and transferred to cooled poured plates of string-bean agar (8 c. c. per plate), to each of which four drops of 20 per cent lactic acid had been added. After several days, a microscopic examination was made of the conidia and mycelium to determine whether other organisms were present. Eight transfers to test-tube slants were made from the margin of several plantings and kept for comparison and for indications of contamination. It may be noted that a large percentage of pure cultures was obtained by this method. From the cultures that were pure, single-spore cultures were obtained according to the method described by Sherbakoff (20, p. 102–103; p. 104, footnote 8). Stock cultures were made from these single-spore cultures and repeatedly repoured to protect from subsequent contamination.

Several species of Fusarium were secured, in order to compare them with the *Fusarium* sp. from the soybean and the one from the cowpea, isolated as described above. The following species, subcultures from Wollenweber's authentic cultures, were obtained through the courtesy of Mr. C. W. Carpenter, of the Bureau of Plant Industry: *Fusarium oxysporum* (Schlecht.), *F. vasinfectum* (Atk.), *F. lycopersici* Sacc., *F. niveum* Smith (members of the section Elegans), and *F. discolor*, var. *sulphureum* (Schlecht.) App. and Wollenw. (1, p. 115–118), (section Discolor).

These species were studied in culture, in order to determine their morphological and cultural characters, since such a study is considered of primary importance in their differentiation. The species mentioned were chosen because all except one belong to the section Elegans, the section which contains the known wilt-producing species, and because, according to Wollenweber, they are the most difficult to separate by this method. *F. conglutinans* Wollenw., *F. redolens* Wollenw., and *F. orthoceras* App. and Wollenw., of the same section, are included in the comparisons. They are so different from the others, as indicated by the

original descriptions, that the writer soon realized that there was little probability of confusing them with the soybean strain. Wollenweber (25, 26) and Sherbakoff (20) have described other species and varieties of the section Elegans which are not, however, included in this study, because they occur on hosts widely separated genetically from the soybean [1] and because the authors have not had opportunity to make a sufficient number of infection experiments to establish them as wilt producers.

CULTURE MEDIA AND THEIR VARIOUS EFFECTS ON SPECIES OF FUSARIUM

In making a cultural study of these fungi much care was taken to follow the suggestions of Appel and Wollenweber (1), Wollenweber (25, 26), and Sherbakoff (20), in order to determine what criteria to employ in judging normal growth characters. It is generally believed that standardization of cultural methods is highly essential in the comparative study of so difficult a group of fungi.

The writer has kept the soybean and the cowpea strains under constant observation for two years on various kinds of "natural and artificial media" and under widely variable physical conditions. He is therefore familiar with the possible variability of members of this genus.

Since a large number of the media used did not prove to be of special diagnostic value, they are not discussed here. Among the media most commonly employed and serving some particular purpose were oat, potato, and string-bean hard agars (3 per cent agar), which, because of the paucity of moisture (20, p. 106), give all forms of fruitification with "normal" spores. Five to 10 per cent of dextrose was added to agars to favor the production of pigment. Growth on steamed rice in test tubes from weighed quantities of rice and measured amounts of water to obtain uniformity also results in the formation of pigment and sometimes an odor that is typical for certain related species of Fusarium. Herbaceous and woody stems, string-bean pods, and potato plugs give the best development of sporodochia and pionnotes.[2] Potato plugs also serve for the proper development of sclerotia and colors, both of which may be reduced or absent from stem plugs when there is a minimum development of mycelium.

According to Wollenweber (25, p. 37), virulence is commonly maintained on stem plugs. Living sterile soybean and cowpea seedlings grown in 6-inch test tubes were also used and are thought to be a better medium for maintaining virulence in the strains from the respective hosts.

[1] Wollenweber, H. W. (25, p. 37) says, "The parasite from one host, as a rule, has not been found on living organs of another host. In pure culture the parasite from one host . . . did not cause wilt in any other host as a result of inoculation experiments."

[2] For a discussion of these terms, see Wollenweber (25, p. 24, footnote).

In order to obtain sterile seedlings for this purpose the seeds were first washed for 5 minutes in tepid water and were then placed in concentrated sulphuric acid for 20 minutes. Formalin, mercuric chlorid, both in aqueous and alcoholic solution, and other disinfectants were employed with much less success. After washing off the acid in three or four changes of sterile water, the seed were transferred into sterilized moist chambers in the bottoms of which several layers of moist filter paper had been placed. Germinated seeds on which there was no evidence of contamination after a day or two were transferred to sterile test tubes [1] the bottom of each of which contained a wad of moistened filter paper.[2] If, during germination or transfer, contamination occurs, it generally becomes evident on the seedlings or white paper, especially if the seedlings are set aside until they have grown to a height of 3 or 4 inches.[3]

METHODS OF STUDY AND PRESENTATION

All transfers of different strains in a set for comparison were made to a certain medium on the same day and to additional media on later days until the set was growing on a sufficient number of media to provide the necessary cultural characters. When species were compared, they were always of the same age and were grown on the same medium. As many comparisons could be made on the same day as there were species and kinds of media in the set. If sufficient data had not been obtained, if certain cultures were abnormal, or if other species or media were to be used, new sets were prepared of all of the species using the desired media and comparisons were again made throughout the series.

Cultural differences also arise as a result of the employment of spores or a bit of mycelium in inoculation. In the former case the young cultures quickly produce spores with a scant mycelial growth, while in the latter the mycelial growth is abundant and there is a paucity of spores. For this reason spores from sporodochia, when present, were used, and in all cases, in so far as was possible, the same kind of inoculum was transferred for all cultures of a set. When the production of spores becomes subnormal, as it often does in cultures, considerable time and patience may be required to bring the strain back to a "*Normkultur*." This was accomplished by transferring a small portion of mycelium to a variety of media until a medium was found on which spores were again obtained.

[1] For making this last transfer, dip the ends of long tweezers into 95 per cent alcohol and ignite in the flame. This sterilizes instruments, burns off the excess of alcohol, and leaves them dry and cool enough for immediate use.

[2] The use of agar as a substratum for this purpose (Garman and Didlake, 7), and Sphagnum moss, did not prove to be satisfactory. Soil, too, has a disadvantage in that it does not show the contaminations as readily as filter paper or agar.

[3] An oat sprouter with glass front, heated by a kerosene lamp and costing about $10, makes a good light incubator for such purposes when the greenhouse is not conveniently located or the temperature suitable. This sprouter is unsuited, of course, to cultures or material requiring a constant temperature.

All cultures were kept in the laboratory at room temperature, 12° to 26° C., and in diffused daylight, so that they were subjected alike to any change of environmental conditions.

In all cases 10 cultures of a species were made on each medium. Different forms of fructification which normally appear on a certain medium may not do so in every tube. For example, in a species where sporodochia are not abundant, they may perhaps form on only 2 or 3 of the 10 stem plugs; or if the form produces green sclerotia, they may develop on not more than 5 of the 10 potato plugs. In some instances as many as 8 to 10 sets of 10 tubes each of a particular species were made.

In making the microscopical examination note was taken of the size, abundance, and type of conidia (fig. 1, *A-G*), chlamydospores, and conidiophores. In measuring spores several fields were first examined to fix in mind the prevailing type and an average of 10 or more of these typical spores was made. Careful note was taken also of extreme types.

In the macroscopic study of the cultures the nature of the stromata, the pionnotes and sporodochia, the character of the aerial mycelium, the color of spore masses, aerial and submerged mycelium and substratum, and the production of sclerotia were considered.

RESULTS OF THE COMPARISON OF THE SOYBEAN FUNGUS WITH OTHER MEMBERS OF THE SECTION ELEGANS

The first sets of parallel cultures were intended to serve in the separation of any or all of the species of Fusarium causing wilt from the soybean fungus. *F. discolor* var. *sulphureum*, *F. oxysporum*, *F. vasinfectum*, *F. lycopersici*, *F. niveum*, *F. tracheiphilum*, and *Fusarium* sp. from soybean were therefore grown on the following media, several sets of 10 cultures of each species being used on each medium: Potato plugs, steamed rice, cotton stems, potato hard agar, and string-bean hard agar. The cultures were examined when 8, 15, 19, 30, and 50 days old. The results are noted in Table I. Only those characters are recorded that are necessary or useful for the separation of the species.

TABLE I.—*Characters which separate a number of the wilt-producing species of Fusarium from F. tracheiphilum and the soybean fungus*

Species.	Sclerotia.	Sporodochia.	Pionnotes.	Chlamydospores.
F. discolor	None	Numerous	Perfect	Intercalary; no measurements.
F. vasinfectum	Green and flesh-colored.	do	do	Intercalary and terminal; no measurements.
F. oxysporum	do	Few	Reduced	Intercalary and terminal; 6 to 12 μ.
F. lycopersici	Flesh-colored	Numerous	Perfect	Intercalary and terminal; no measurements.
F. niveum	Large green	do	Reduced	Same as for *F. lycopersici*.
F. tracheiphilum	Green and flesh-colored.	Few	None	Intercalary and terminal; 6 to 12 μ.
Fusarium sp. on soybean	Mostly green; some flesh-colored.	do	do	Same as for *F. tracheiphilum*.

TABLE I.—*Characters which separate a number of the wilt-producing species of Fusarium from F. tracheiphilum and the soybean fungus*—Continued

Species.	Macroconidia.		Odor.
	Size of 3-septate.	Type.	
F. discolor	No data	Discolor; mostly 5-septate.	None.
F. vasinfectum	Same as in *F. oxysporum*	Elegans; mostly 3-septate.	Strong lilac on rice.
F. oxysporum	28.7 to 35.6 by 3.6 to 4.1 μ	do	Often none, sometimes scant lilac.
F. lycopersici	Abnormal	do	None.
F. niveum	Abnormal; (original description gives larger than in *F. Oxysporum*).	do	Do.
F. tracheiphilum	23.6 to 41.0 by 3.9 to 4.1 μ	do	Do.
Fusarium sp. on soybean	24.6 to 35.8 by 2.89 to 4.1 μ	do	Do.

From the data in Table I it is important to observe that *F. tracheiphilum* and the species of Fusarium on soybean belong to the section Elegans, as established by Appel and Wollenweber (1) and modified by Wollenweber (25) in a subsequent study. They are themselves very similar in cultural characters, but can be quite sharply separated from the other species included in the tabulation. When the characters of the species of Fusarium on the cowpea and soybean noted in this table are compared with those in the original descriptions of certain other members of the section Elegans—namely, *F. redolens*, *F. orthoceras*, and *F. conglutinans*, there is plainly no chance of their confusion. *F. redolens* (25) produces no blue sclerotia, and its conidial masses are brownish white; *F. orthoceras* (25) possesses neither sclerotia, sporodochia, nor pionnotes; and *F. conglutinans* (25) is distinguished because of the absence of the typical wine-red to purple colors of the section.

MORPHOLOGICAL AND CULTURAL COMPARISON OF THE FUSARIUM SP. ON SOYBEAN WITH F. TRACHEIPHILUM

Since the studies summarized in Table I do not succeed in distinguishing the species of Fusarium on soybean and cowpea, a more extensive cultural study of these two fungi was made. For this purpose three series of cultures were grown, and the results have been summarized in Table II. Each series contained 10 cultures of each fungus on stem plugs, potato plugs, steamed rice, standard nutrient agar (1.8 per cent agar and 1.0 per cent acid), string-bean hard glucose agar (3 per cent agar, 1.0 per cent acid, and 10 per cent glucose), and oat hard agar (3 per cent agar and 1.0 per cent acid). The cultures were examined when they were 8, 15, 30, 50, and 75 days old.

TABLE II.—*A morphological comparison of the species of Fusarium on soybean and cowpea*

FUSARIUM SP. ON SOYBEAN

Medium.	Macroconidia.	Sporodochia.	Sclerotia.	Color of mycelium.	Character of mycelium.
Standard nutrient agar.	No measurements.	Salmon-colored..	None.....	White.....	Mostly aerial and floccose, becoming appressed in old age.
String-bean agar.....	.....do	Salmon-colored; generally present.	Green.....	.....do.....	Do.
Oat hard glucose agar.	26.6 to 38.6 by 3.69 to 4.92 μ 50 days old.	Flesh-colored....	Dark green..	Mostly lilac; some dark purple.	Cottony.
Steamed rice.....	.....	.....	.....	Reds, pinks, lilacs, purples.	
Potato plugs.....	Normal spores absent.	Salmon-colored; generally present on sclerotia.	Dark green..	Green near sclerotia.	Floccose.
Stem plugs.....	22.5 to 43.6 by 2.87 to 4.11 μ 14 days old.	Salmon-colored; small.	Green; very small; numerous.	White; sometimes green near sclerotia.	Floccose; scant.

F. TRACHEIPHILUM

Standard nutrient agar.	.....	None.....	Flesh-colored..	White.....	Mostly submerged or appressed.
String-bean agar.....	No measurements.	Salmon-colored; few.	Mostly flesh-colored; some green.	.....do.....	Do.
Oat hard glucose agar.	22.5 to 36.9 by 3.8 to 4.42 μ 50 days old.	Flesh-colored..	Dark green and flesh-colored.	Mostly dark purple; some lilac.	Cottony to matted and appressed.
Steamed rice.....	.....	.....	.....	Pinks, reds, lilacs, purples.	
Potato plugs.....	24.6 to 36.9 by 3.28 to 4.42 μ 19 days old.	Salmon-colored; often on sclerotia.	Flesh-colored; often none.	Pinks, lilacs, greens.	Mostly appressed.
Stem plugs.....	.....	Salmon-colored; small; sometimes none.	Green; very small; numerous.	White; sometimes green near sclerotia.	Appressed good growth

No mention is made in Table II of pionnotes or odors, as none were produced in any of the cultures. The microconidia of both strains show a wide variation both in size and shape, but these differences can properly be included in the range of variation. The normal macroconidia of the soybean (fig. 1, *A–G*) and cowpea strains are indistinguishable. The chlamydospores of either strain are terminal or intercalary in or on vegetative filaments and average 6 to 10.25 μ in diameter. The conidiophores are verticillately branched when normal. Sporodochia, although sometimes flesh-colored, are normally salmon-colored. They are not always present on all media, but are formed by each strain either on sclerotia or on mycelia as stromatal bases. Green sclerotia are normally present in both strains. There appear to be some differences in colors produced in substrata, although not very consistent ones, a difference in the character of mycelium until advanced ages of the cultures and gen-

erally, but not always, an absence of flesh-colored sclerotia in the soybean fungus. These differences, however, are not believed to be of sufficient importance to warrant regarding the soybean strain as a distinct species or variety.

In addition to the media employed in Table II, potato hard agar, cornmeal plugs, and string-bean pods were used; but they showed no additional characters of value.

Perithecia have never been observed on the diseased stems; neither have they been obtained in cultures from the surface spores nor from the diseased internal tissues. In fact, the cultural differences between the *Fusarium* sp. on soybean and *Neocosmospora* spp. are as striking as between *Neocosmospora* spp. and the several species of Fusarium causing wilt studied by Higgins (12) and Butler (3).

INOCULATION EXPERIMENTS

From the foregoing morphological and cultural studies, it is evident that the species of Fusarium on soybean is not distinguished from *F. tracheiphilum* by any well-defined differences. Since the possibility existed that they might be separated by biological differences, reciprocal inoculation studies were undertaken to secure additional evidence of their identity.[1]

Plants were therefore grown in pots and flats in the greenhouse and in plots in the field for use in inoculations. The soil used in the pots and flats was a fine, compact, sandy loam, except in the case of one experiment, and was taken from a field in which diseases of cowpeas and soybeans caused by *Fusarium* spp. had never been observed. In certain of these tests, as an added precaution, the soil was partially sterilized by the use of a 2 per cent solution of formaldehyde. The seed were also sterilized in certain experiments by immersion for 15 minutes in commercial sulphuric acid. Since uninoculated plants remained free from disease when these precautions were not employed, their use was discontinued in subsequent tests.

The pots and flats were of sufficient size to permit the plants to grow to maturity.

In determining the percentage of diseased plants, count was made only of those in which it was possible to find discoloration and invasion of the xylem tissues. In case of doubt in this microscopic examination, planted plates were made from the tissues and the subsequent growths studied.

The varieties of soybeans and cowpeas planted for the cross-inoculation experiments were known to be subject in the field to the species of Fusarium on soybean and cowpea, respectively.

EXPERIMENT I.—Twenty-five North Carolina Black cowpea and 25 Mammoth Yellow soybean seedlings, growing in each of two flats

[1] Wollenweber (25, p. 37) says that a consideration of the biological characters is of secondary importance in the determination of species.

in the greenhouse, were each inoculated when from 3 to 6 inches high with spores from sporodochia and with mycelium by introducing the material into incisions in the stems an inch or two below the surface of the soil. All of the plants in one flat were inoculated with the soybean strain of Fusarium and all of those in the other with the cowpea strain. Checks and all inoculated plants except two cowpeas inoculated with the soybean strain and one with the cowpea strain, remained healthy. The test was repeated, using a freshly isolated strain of both organisms; and, since all but one of the plants remained healthy, this method of inoculation was discarded.

EXPERIMENT II (Series 1).—In this experiment the soil in two flats A and B, in the greenhouse was inoculated with pure cultures of *Fusarium* spp. on cowpea and soybean, respectively. These cultures were then incorporated in the upper 4 inches of soil.

The organisms had been grown on pieces of moistened, sterilized cowpea stems until numerous sporodochia had formed. On April 12, 1916, 20 North Carolina Black cowpeas and 20 Mammoth Yellow soybeans were planted in each flat. A third flat, containing uninoculated soil, was planted as a check.

By June 4 a cowpea plant in flat B was noted to be diseased. Others had been observed to be affected by June 15, when all the plants were removed and examined. The results are presented in Table III.

TABLE III.—*Results of growing soybeans and cowpeas in artificially inoculated soil*

Flat.	Organism.	Host.	Total number of plants.	Diseased plants.	
				Number.	Percentage.
A	*Fusarium* sp. on cowpea	Cowpeas	20	6	30
		Soybeans	20	3	15
B	*Fusarium* sp. on soybean	Cowpeas	20	10	50
		Soybeans	20	7	35
C	None (control)	Cowpeas	20	0	0
		Soybeans	20	0	0

EXPERIMENT II (Series 2.)—Since the percentage of diseased plants in series 1 is relatively small, the test was repeated, using another strain of each organism and Clay cowpeas instead of the North Carolina Black variety. Each plant in this test was injured by incision below the surface of the inoculated soil. The period of growth of these plants extended from July 29 to September 1, at which date the plants were fully matured. The results of this series are recorded in Table IV.

EXPERIMENT II (Series 3).—The test in series 2 was duplicated between September 7 and November 20, with no resultant increase in the percentage of infections.

EXPERIMENT III.—Since it was thought that the strain of Fusarium on soybean had to a degree lost its virulence by growth in culture, soy-

bean stems bearing an abundance of sporodochia were macerated and mixed with the soil in two flats. Seed of the Mammoth Yellow variety were planted on May 25. When the experiment was concluded, August 10, only 8 of the 80 soybean plants in these two flats were found to be infected.

TABLE IV.—*Results of growing soybeans and cowpeas in artificially inoculated soil, the plants having been wounded below the surface of the soil*

Flat.	Organism.	Host.	Total number of plants.	Diseased plants.	
				Number.	Percentage.
D	*Fusarium* sp. on cowpea	Cowpeas	20	3	15
		Soybeans	20	3	15
E	*Fusarium* sp. on soybean	Cowpeas	20	6	30
		Soybeans	20	5	25
F	None (control)	Cowpeas	20	0	0
		Soybeans	20	0	0

EXPERIMENT IV.—This experiment was made with soybeans between September 26 and December 1 in an attempt to determine whether the presence of nematodes increases the number of infections. The nematodes were introduced into the soil of large buried pots in root galls from living soybeans free from infection by *Fusarium* sp. The results are presented in Table V.

TABLE V.—*Influence of nematodes on the percentage of infection of soybeans with species of Fusarium*

Organism.	Total number of plants.	Number with nematode galls.	Number with *Fusarium* sp.
Fusarium sp. on cowpea and nematodes	10	10	2
Fusarium sp. on cowpea without nematodes	10	0	0
Fusarium sp. on soybean and nematodes	10	10	3
Fusarium sp. on soybean without nematodes	10	0	2
Nematodes only	10	10	0
None (control)	20	0	0

Only one test of this kind was made; and it is significant to note that there was no increase in infection by the fungus, although the plants in all of the pots into which the galls had been introduced were attacked by eelworms. This experiment was concluded before the plants had matured.

EXPERIMENT V.—Since the more porous types of sandy soil have generally been observed to favor the development of *Fusarium* spp., the cause of wilt diseases, an experiment was performed which was in duplication of Experiment II, series 1, except that the soil consisted of a mixture of three parts of medium-coarse sand and one part of fine sandy

loam and stable manure. The results obtained between September 7 and the close of the experiment, November 20, show considerable increase in the percentage of infection over those in the more compact, fine sandy loam of the preceding experiments even though the cultures used were transfers from cultures nearly 3 months old. Cultures of the strain of *Fusarium* on cowpea were added to flat G, of the species of *Fusarium* on soybean to flat H, and flat J was held as a control. Table VI contains the data on this experiment.

TABLE VI.—*Influence of soil on percentage of infection by Fusarium spp.*

Flat.	Organism.	Host.	Total number of plants.	Diseased plants.	
				Number.	Percentage.
G	*Fusarium* sp. on cowpea	Cowpeas	20	16	80
		Soybeans	20	12	60
H	*Fusarium* sp. on soybean	Cowpeas	20	13	65
		Soybeans	20	12	60
J	None (control)	Cowpeas	20	0	0
		Soybeans	20	0	0

EXPERIMENT VI.—This experiment was designed to confirm the results of inoculations in the greenhouse by inoculations under partially controlled field conditions. Four small plots (No. 26, 27, 28, and 29) on wilt-free soil of the station farm were inoculated as in the previous experiments with pure cultures of the species of Fusarium on soybean; plots No. 59 and 60 were inoculated with the *Fusarium* sp. on cowpea; and two others (100 and 101) were left untreated as controls. Thirty cowpeas and thirty soybeans were planted in each plot on June 10, and the final results noted in Table VII were obtained on September 4.

TABLE VII.—*Results of cross-inoculations in the field*

Plot No.	Organism.	Host.	Total number of plants.	Diseased plants.	
				Number.	Percentage.
26	*Fusarium* sp. on soybean.	Clay cowpeas	30	17	56.6
		Haberlandt soybeans	30	4	13.3
27	.....do.....	Clay cowpeas	30	10	33.3
		Tokio soybeans	30	0	0.0
28	.....do.....	Clay cowpeas	30	10	33.3
		Mammoth Yellow soybeans	30	8	26.6
29	.....do.....	Clay cowpeas	30	15	50.0
		Tar Heel Black soybeans	30	3	10.0
59	*Fusarium* sp. on cowpea.	Clay cowpeas	30	26	86.6
		Tokio soybeans	30	6	20.0
60	.....do.....	Clay cowpeas	30	17	56.6
		Mammoth Yellow soybeans	30	6	20.0
100	None (control)	Clay cowpeas	30	0	0.0
		Mammoth Yellow soybeans	30	0	0.0
101	.....do.....	Clay cowpeas	30	0	0.0
		Mammoth Yellow soybeans	30	0	0.0

EXPERIMENT VII.—On May 25, 1916, two 100-foot rows of each of the soybean varieties Tokio, Haberlandt, Mammoth Yellow, Medium Yellow, and Virginia were planted in a field which produced a large percentage of wilt in cowpeas in 1914. Two rows of cowpeas were planted in the same plot. By September 1, when all the plants had fully matured, a small percentage of wilted cowpeas had been noted; but no blighted soybeans were found.

Similar data were obtained from observations on cowpeas and soybeans grown in the experimental plot devoted to plant breeding. In this 4-acre plot, three or four rows of soybeans were alternated with three or four rows of cowpeas throughout the field. Some wilt occurred in practically every row of cowpeas in the plot, but careful examinations during the season failed to reveal a single soybean blighted with *Fusarium* sp. among 17 standard varieties and 50 other unnamed selections.

EXPERIMENT VIII.—The field at Red Springs, N. C., in which at least 60 per cent of the Mammoth Yellow soybeans were blighted in 1915, was again planted with this variety on May 23, 1916. In a portion of the field which had been reserved for the purpose, one 54-meter row each of Haberlandt, Mammoth Yellow, Pekin, Black Eyebrow, Medium Yellow, and Tar Heel Black soybeans and a row of Clay cowpeas were planted on June 8. On August 10 the main field and all of the varieties in the test, including the cowpeas, showed considerable blight or wilt, except the Black Eyebrow and the Virginia varieties of soybeans. On August 26 the latter of these varieties was apparently free from disease, but the plants had declined with age to such an extent that the exact determination was doubtful. The Black Eyebrow variety, however, remained free from disease throughout the season.

SUMMARY

(1) A disease of soybeans not previously reported has been studied during the past two years.

(2) The disease is characterized by a chlorosis and shedding of the leaves or leaflets, followed by the death of plants, and is herein called "blight or wilt."

(3) Soybean-blight has been observed in several localities within North Carolina on soils infected with cowpea-wilt.

(4) A species of Fusarium belonging to the section Elegans is the causal organism.

(5) Cultural and morphological studies which are regarded as of primary importance in distinguishing species of Fusarium show that the strain of Fusarium on soybean is identical with the organism producing the wilt of cowpeas.

(6) Reciprocal inoculation experiments with the strains from soybeans and cowpeas show that cross-inoculations can be made. These experiments were conducted in the greenhouse and under field condi-

tions. Pure cultures of the two strains were used in certain of the experiments and inoculum from the natural host in others.

(7) Infection probably occurs through the roots, but nematodes appear not to increase the percentage of blight materially.

(8) The character of the soil appears to influence the percentage of infection, since the largest proportion of diseased plants appeared in coarse sandy soil.

(9) Blight or wilt of soybeans is therefore due to *Fusarium tracheiphilum* Smith.

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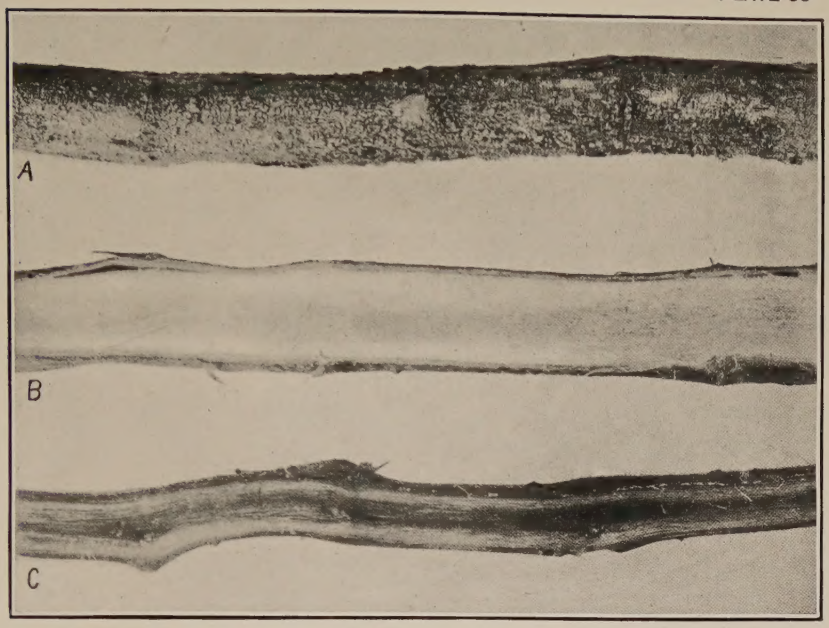
PLATE 95

A.—A diseased stem of soybean, showing the roughened appearance caused by the irregular covering of sporodochia.

B.—Interior of healthy (unstained) stem of soybean.

C.—Interior of diseased (discolored) stem of soybean.

D.—Soybean plants grown out of doors in the same type of clay soil: *D*, healthy; *E*, diseased through the naturally infected soil.


A
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